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APPLICATION NUMBER: ***60/042,911***
FILING DATE: ***February 20, 1997***

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

N. WOODSON
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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Attn: Box Missing Parts, Washington, D.C. 20231, on

August 22, 1997

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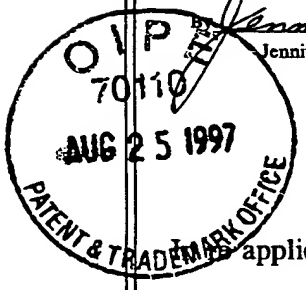
By Jennifer K. Hardin



PATENT

Attorney Docket No. 018148-000100

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	
David S. Dime et al.	Examiner: Unassigned
Serial No.: 60/042,911	Art Unit: Unassigned
Filed: February 20, 1997	TRANSMITTAL LETTER - RESPONSE TO NOTICE OF MISSING PARTS
For: ORALLY ACTIVE SITE-SPECIFIC DRUG DELIVERY	

Attn: Box Missing Parts
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Pursuant to the Notice to File Missing Parts of Application - Filing Date Granted dated July 2, 1997, enclosed is the following to be made of record in the above-identified application:

1) Provisional Application cover sheet identifying city and state of the residence of each inventor
2 Copy of Notice of Missing Parts

Please charge Deposit Account No. 20-1430 for the following fees:

(a) Filing Fee	$ 150.00
(c) Missing Parts Surcharge (§ 1.16(l))	$ 50.00
TOTAL FEES TO BE CHARGED	**$ 200.00**

The Commissioner is hereby authorized to charge any additional fees associated with this paper or during the pendency of this application, or credit any overpayment to Deposit Account No. 20-1430 for this paper and during the prosecution of this application. This Transmittal Letter is submitted in triplicate.

Respectfully submitted,

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02/20/97

PROVISIONAL APPLICATION

Atty. Docket No. 018148-000100
"Express Mail" Label No. EM530421147US
Date f Deposit FEBRUARY 20, 1997

I hereby certify that this is being deposited with the Postal Service "Express Mail Post Office to Address" service under 37 CFR 1.10 on the date indicated above addressed to the Asst. Commissioner of Patents, Box Provisional Patent Appln., Washington, DC 20231.

By: Robert Bell
Robert Bell

60/042911
02/20/97
71622 U.S. PTO

BOX PROVISIONAL PATENT APPLICATION
ASST. COMMISSIONER OF PATENTS
Washingt n, D. C. 20231

Sir:

Transmitted herewith for filing is a provisional patent application under 37 CFR 1.53(b)(2) of:

LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY/STATE/COUNTRY)
Dime	David	S.	Canada
Backx	Peter		Canada

Title: ORALLY ACTIVE SITE-SPECIFIC DRUG DELIVERY

Enclosed are:
[X] 21 pages of the specification (including description and claims).
[X] 2 sheet(s) of drawing(s).
[X] Abstract
[ ] A verified statement to establish small entity status under 37 CFR 1.9 and 37 CFR 1.27.
[ ] The invention was made by or under a contract with the following agency of the United States Government: _____ under Government contract number: _____.
[ ]
Please charge Deposit Account No. 20-1430 as follows:
[X] Filing fee $_____
[X] Any additional fees associated with this paper or during the pendency of this application.

2 extra copies of this sheet are enclosed.

Respectfully submitted,

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PROVISIONAL PATENT APPLICATION

ORALLY ACTIVE SITE-SPECIFIC DRUG DELIVERY

Inventors:

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AS FILED FEBRUARY 20, 1997

[redacted]

PATENT

Attorney Docket No. 18148-000100

ORALLY ACTIVE SITE-SPECIFIC DRUG DELIVERY

FIELD OF THE INVENTION

The present invention relates to new methods of drug delivery using modified therapeutic agents. In particular, it relates to the preparation of drug having an attached targeting anchor and methods of delivering the drug to a predetermined site.

BACKGROUND OF THE INVENTION

The specific targeting of drugs to a receptor site is extremely advantageous, particularly in the case of toxicity or limited specificity. Linked drugs, in the form of immunoconjugates, have been used to assist in targeted drug delivery (examples of this: B.A. Froesh, R. A. Stahel, W.U. Zangmeister, *Cancer Immunol. Immunother.*, **42** 55 (1996); D. Willner, P.A. Trail, S.J. Hofstead, H.D. King, S.J. Lash, G.R. Braslawsky, R.S. Greenfield, T. Kancko, R.A. Firestone, *Bioconjugate Chem.* **4** 521 (1993)). These strategies have severe limitations as many cellular sites cannot be targeted with immunoconjugates. Moreover, immunoconjugates are delivered by injection, thus limiting their widespread use.

What is needed in the art are new compounds and methods for the active site-specific delivery of pharmaceutical agents. The new compounds should provide alternatives to immunoconjugates by having targeting or anchoring portions which are not antibody-based. Quite surprisingly, the present invention provides such compounds and methods of delivery.

SUMMARY OF THE INVENTION

The present invention provides compounds and methods which are useful for the site-specific delivery and localization of drugs. The compounds can be represented by the formula:

A—L—D

wherein A is an anchoring moiety; L is a linking group; and D is a drug. In preferred embodiments, the anchoring moiety is a functional group capable of covalent attachment to a target site. Particularly preferred are those anchoring moieties having a sulfhydryl-reactive group (*e.g.*, alkanethiosulfonate esters, dithiopyridyl groups, maleimide and cystine. Other particularly preferred embodiments are those in which the anchoring moiety is a reactive functional moiety, for example, an α-diazo ketone, α-halo ketone, pentafluorophenyl ester, or 2,4-dinitrophenyl ester. In another group of embodiments, the anchoring moiety is a non-peptide affinity ligand for a target site.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1a and 1b illustrate two binding models for the targeted agents of the present invention.

Figure 2 provides the structures of several compounds of the present invention, including C_2, C_6, C_{10} and T_2.

DETAILED DESCRIPTION OF THE INVENTION

General

In order to develop generally useful agents that specifically target one receptor site, while excluding other tissues, we describe herein a novel paradigm for drug design as illustrated in Figure 1a. According to this figure, the compounds are comprised of three functional domains; an

anchor, a linker and an active agent (i.e. protein modifying motif, active portion of the drug). As used herein, the compounds containing the three functional domains will be referred to as "targeted agents." The key to this novel method of drug design is the anchor which recognizes a distinct molecular target (*i.e.*, protein) in a specific tissue. As a result, the anchor effectively delivers the active agent to the specific tissue/target. This model is equally applicable for the targeting of drugs to other tissues.

Furthermore this strategy can take advantage of both the anchor and the active portion of the drug in a synergistic relationship to enhance local concentration at the desired site. For example, for those therapeutic agents or drugs which exhibit an inherent tendency to accumulate at a desired site, the use of a suitable anchor will further maintain the drug at the site and provide an apparent synergistic effect.

A number of proteins are expressed in the body in tissue specific manners. Accordingly, anchors can be designed for targeting protein modifiers to many tissues, based on detailed molecular structural information derived from published x-ray structures of proteins. It is important to note that the regions of the protein which interact with an anchor do not need to be located immediately adjacent to the region of the protein whose function is altered by the drug. Indeed, the anchor and active agent might be located on different proteins.

As noted above, it is not necessary for the anchor and therapeutic agent to bind to the same protein, provided the linking group between the anchor and therapeutic agent is sufficiently long to allow both groups to interact with their target sites. For example, cellular proteins include membrane proteins which are often co-localized into functional domains of interacting proteins. Thus, certain compounds of the present invention could have binding portions (anchoring groups and therapeutic agents or drugs) which bind to separate sites in the interacting proteins.

Still further, it is not necessary that the anchor

be inert (as to pharmacological effects). For example, the anchor can itself be a therapeutic agent which acts on one site, while the attached drug interacts at another site in close proximity.

This technology could be applied in order to deliver virtually any drug.

Embodiments of the Invention

In one aspect, the present invention provides modified therapeutic agents which have an anchoring or targeting portion specific for a particular site or tissue. The modified therapeutic agents are represented by the formula:

A–L–D

in which A is an anchoring group, L is a linking group and D is a drug.

Anchoring groups which are useful in the present invention are those groups which will bind to a particular tissue or protein of interest in either a tight-binding non-covalent manner or in a covalent or irreversible manner. The anchoring groups can be either targeting groups or simple functional groups which exert their "anchoring effect" once in position. In the case of targeted anchors, the anchor will typically be a group which acts as an affinity ligand for a specific tissue, protein or binding site. Affinity ligands are well known to those of skill in the art and include certain modified peptides or small modified proteins which have been altered to have reactive functional groups such as, for example, α-halo ketones, α-diazo ketones, or activated ester groups (*e.g.*, 2,4-dinitrophenyl esters or pentafluorophenyl esters). As noted above, however, covalent attachment to a target site is not a required property for the compounds of the present invention. Non-covalent anchoring can take place via suitable electrostatic interactions with, for example, ammonium ion groups present in the target tissue and carboxylic acid groups present in the anchoring portion. In the case of simple anchors which bind covelently to a site

at or near a targeted tissue, effective targeting will still be accomplished via attachment of a drug which is known or predicted to localize or accumulate at the selected tissues. Examples of suitable covelent anchors include sulfhydryl-reactive groups (*e.g.*, methanethiosulfonyl groups, dithiopyridyl groups, other reactive dissulfides, and cystine), alkylating agents (*e.g.*, α-halo ketones, α-diazo ketones), and acylating agents (*e.g.*, activated esters such as 2,4-dinitrophenyl esters and pentafluorophenyl esters, and certain anhydrides). Still other suitable anchoring groups are known to those of skill in the art.

Linking groups or spacers which are useful in the present invention are those groups which are inert to proteolytic or other degredative processes in the body. The linking groups will typically be alkylene chains, aryl acetylenes, ethylene glycol oligomers containing 2-14 monomer units, diamines, diacids, or combinations thereof. In preferred embodiments, the linking group is an alkylene chain of from two to 24 carbon atoms, more preferably from two to ten carbon atoms. The linking groups, L will typically have a a functional group (*i.e.*, hydroxyl, amino or carboxylic acid) at each terminus for the attachment of the anchoring or targeting moiety and for the attachment of the drug.

The conventional drugs used in the present invention can be any of a variety of drugs which are selected to be an appropriate treatment for the disease to be treated in the targeted tissue. Often the drug will be an antineoplastic agent, such as vincristine, doxorubicin, cisplatin, bleomycin, cyclophosphamide, methotrexate, streptozotocin, and the like. It may also be desirable to deliver anti-infective agents to specific tissues by the present methods. The present inventive method can also be used for the selective delivery of other drugs including, but not limited to local anesthetics, *e.g.*, dibucaine and chlorpronazine; beta-adrenergic blockers, *e.g.*, propranolol, timolol and labetolol; antihypertensive agents, *e.g.*, clonidine and hydralazine; anti-depressants, *e.g.*, imipramine, amitriptyline and doxepim; anti-convelsants, *e.g.*, phenytoin; antihistamines, *e.g.*,

diphenhydramine, chlorpheniramine and promethazine; antibacterial agents, *e.g.*, gentamycin; antifungal agents, *e.g.*, miconazole, terconazole, econazole, isoconazole, butaconazole, clotrimazole, itraconazole, nystatin, naftifine and amphotericin B; antiparasitic agents, hormones, hormone antagonists, immunomodulators, neurotransmitter antagonists, antiglaucoma agents, vitamins, narcotics, and imaging agents. Other particular drugs which can be selectively administered by the method of the present invention will be well known to those of skill in the art. Additionally, two or more therapeutic agents may be administered simultaneously if desired, where such agents produce complementary or synergistic effects.

Preparation of the above compounds can be carried out by standard synthetic methods (see, March, ADVANCED ORGANIC CHEMISTRY, 4TH ED., Wiley-Interscience, New York, 1992). For example, a suitable linking group with functionality (*e.g.*, hydroxyl groups, thiols, carboxylic acid or amines) at each terminus can be mono-protected to provide a single reactive functionality. Reaction of the single functionality with a suitable therapeutic agent provides a therapeutic agent with a covalently bound linking group (for example, via an ester, amide, disulfide, ether or other similar linkage). Subsequent deprotection at the distal end of the linking group and attachment of the anchoring portion provides the compounds of the present invention.

In another aspect, the present invention provides methods of localizing a therapeutic agent in a preselected tissue. According to these methods the compounds provides above are administered to a suitable host. Preferred hosts include mammalian species, such as humans, non-human primates, dogs, cats, cattle, horses, sheep, and the like.

The targeted drugs may be contacted with the target tissue by a variety of methods. Generally, the contact will be made by direct application of the targeted drugs to the tissue. The application may be made by topical, "open" or "closed" procedures. By "topical", it is meant the direct application of the targeted drugs to a tissue exposed to the

environment, such as the skin, oropharynx, external auditory canal, and the like. "Open" procedures are those procedures include incising the skin of a patient and directly visualizing the underlying tissue to which the targeted drugs are applied. This is generally accomplished by a surgical procedure, such as a thoracotomy to access the lungs, abdominal laparotomy to access abdominal viscera, or other direct surgical approach to the target tissue. "Closed" procedures are invasive procedures in which the internal target tissues are not directly visualized, but accessed via inserting instruments through small wounds in the skin. For example, the targeted drugs may be administered to the peritoneum by needle lavage. Likewise, the targeted drugs may be administered to the meninges or spinal cord by infusion during a lumbar puncture followed by appropriate positioning of the patient as commonly practiced for spinal anesthesia or metrazamide imaging of the spinal cord. Alternatively, the targeted drugs may be administered through endoscopic devices. Preferably, the targeted drugs will be administered orally.

Without intending to bound by any particular theory or method of attachment, a detailed model is provided below for compositions which are useful in the treatment of ventricular and atrial arrhythmias.

Myocardial Ischemia, Arrhythmias and Death

Ventricular and atrial arrhythmias are routinely observed in diseased myocardium and are the leading cause of death (i.e., 50-80%) in patients with acute myocardial infarction, cardiomyopathic and cardiac hypertrophy. In acute myocardial infarctions (i.e., heart attacks) the electrical properties of the ischemic region of the heart commonly result in the development of life-threatening arrhythmias often resulting in death. Furthermore, unsustained ventricular tachycardia in patients with previous myocardial infarctions is closely associated with a two-year mortality rate of approaching 30%. Clearly, targeting therapies at patients with high risk of either acute ischemic events or subsequent

infarction related arrhythmias would be of tremendous benefit for an enormous patient population (estimated to be between 2 and 3 million in the U.S.). The present invention, therefore, provides methods to develop cardiac specific agents for the prophylactic treatment of patients at risk for myocardial ischemia and related arrhythmias.

The strategy used to treat cardiac arrhythmias involves the alteration of cardiac electrical activity through modulation of ion channels. Many therapies have been tested in the treatment of ischemia related arrhythmias and death. Clinical trials with class 1c (i.e., combined Na^+ channel blockers/K^+ channel blockers) and class III (i.e., purely K^+ channel blockers) have proven to be disappointing and, in fact, dangerous. In a number of animal models of acute cardiac ischemia, the most effective agent against the development of ventricular tachycardia and fibrillation has been the type Ib class of drugs (i.e., primarily Na^+ channel blockers). Previous clinical trials with representative agents of this class of drugs (i.e., mexiletine) have not been viable because of toxicity unrelated to the heart. Indeed, nerve and skeletal muscle Na^+ channels, while distinct gene products, are structurally very similar to heart Na^+ channels making them equally good targets for class 1b drugs. Clearly, it would be highly desirable to develop class 1b agents that target specifically and exclusively cardiac Na^+ channels. In view of the molecular differences in Na^+ channel structure for Na^+ channel derived from different tissue combined with the observation that cardiac Na^+ channels are absolutely unique to the heart, the present invention provides class 1b antiarrhythmic agents that are absolutely cardiac specific.

Our Specific Strategy for Targeting Antiarrythmics to the Heart

Our first prototype for these drugs is shown in Figure 1B. This agent contains an anchor which is comprised of a sulfhydryl reactive group linked through a hydrocarbon chain (i.e., ethyl group, $(CH_2)_2$) to a class 1b local anesthetic active site. Benzocaine is a prototypic class Ib

agent with very rapid kinetics for binding to Na^+ channels which is non-tissue specific since it binds equally to Na^+ channels throughout the body. We previously established (Backx, *et al.*, *Science* 257:248-251, 1992) that heart Na^+ channels (in all species from which the amino acid sequence is available) have a free reduced sulfhydryl group in the channel pore (i.e., a cysteine residue in the pore) which is not present in other tissue-specific Na^+ channels. Another unique feature of the presence of a free sulfhydryl in heart Na^+ channel pore is the observation that this reactive sulfhydryl is located on the extracellular face of the pore; extracellular sulfhydryls are generally found in an oxidized state usually in the form of a disulfide linkage. Clearly, the presence of this reactive sulfhydryl group, unique to heart Na^+ channels provides a strong rationale for designing compounds with a sulfhydryl reactive anchor. As shown in Figure 1b, compounds which specifically bind to heart tissues and more specifically heart Na^+ channels are now available. The decision to attach a class 1B agent as the reactive portion of the drug was based on two experimental findings. First, the binding site for the group Ib agents was found by Catterall and coworkers to reside on the inner face of the Na^+ channel pore in a region which molecular models suggested was extremely close to the reactive sulfhydryl group, making it unnecessary to provide a long linker portion of the drug. Second, cardiac specific targeting of group 1B agents are of enormous clinical interest.

Methanethiosulfonates as Anchors for Site-Specific Drug Delivery to Cardiac Sodium Channels

Disulfide bonds are a useful method of linking drugs to proteins (see, D. Willner, P.A. Trail, S.J Lash, G.R. Braslawsky, R.S. Greenfield, T. Kaneko, R.A. Firestone, *Bioconjugate Chem.* **4** 521 (1993)).

A number of reagents are available for chemical modification of cysteine sulfhydryl groups in proteins. The most useful of these are the thiosulfonates (see, T.W. Bruice and G.L. Kenyon, *J. Protein Chem.*, **1** 47 (1982)), due to the

rapid reaction of thiosulfonates with thiols under physiological conditions. Others have reported (M.H. Akabas, D.A. Staufer, M. Xu, and G. Yellen, *Neuropharmacology*, **35** 797 (1996); T. Kuner, L.P. Wollmuth, A. Karlin, *Science*, **258** 307 (1992); D.A. Stauffer, and A. Karlin, *Biochemistry*, **33** 6840 (1994)) the development of three charged methanethiosulfonate reagents that have been used extensively to derive structural features of channel proteins (see also, M. Holmgren, Y. Liu, Y. Xu, and G. Yellen, *Neuropharmacology*, **35** 797 (1996); T. Kuner, L.P. Wollmuth, A. Karlin, P.H. Seeburg, and B. Sakmann, *Neuron*, **17** 343 (1996)), and binding site topology (see, D. Fu, J.A. Ballesteros, H. Weinstein, J. Chen, and J.A. Javitch, *Biochemistry*, **35** 11278 (1996)). Methane thiosulfonates were, therefore, chosen as a useful example to illustrate the present invention.

Other examples of the present invention include the combination of sulfhydryl anchors with dihydropyridine Ca^{+2} channel blockers. Previous results in cardiac myocytes have demonstrated that sulfhydryl reactive agents bind to cardiac L-type calcium channels. In addition, with a linker of suitable length, the dihydropyridines could be linked to the cardiac Na^{+} channels and modify adjacent cardiac Ca^{+2} channels. Thus, the present invention provides compounds and methods which can be used to specifically target the heart with Ca^{+2} channels blockers which are receiving considerable attention in the long-term treatment of heart disease and heart failure.

Still other examples of the present invention include the use of β-antogonists as anchors linked to modifiers of Na^{+}, Ca^{+2} or K^{+} channels. Currently available β-antagonists (or β-blockers) bind to the β-receptors with very high affinities and are uxtensively used in the treatment of acute and chronic heart failure. Attaching a channel modifier provides useful compounds for co-localizing the channel modifying compounds along with β-blocking compounds to the heart.

Yet another example is the combination of FK-binding protein drugs with inhibitors of SR Ca^{+2}-release channels for

the treatment of malignant hyperthermia.

The following examples are offered solely for the purposes of illustration, and are intended neither to limit nor to define the invention.

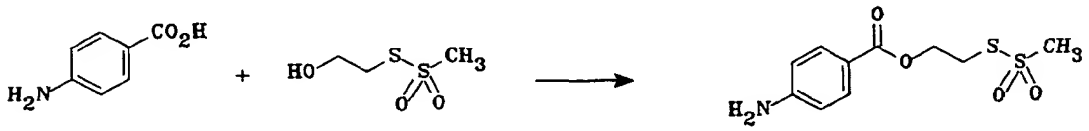
EXAMPLES

Materials

Unless otherwise noted, all reagents are commercially available from such suppliers as Aldrich Chemical Co. (Milwaukee, Wisconsin, USA). Abbreviations are used for common solvents and certain well-known reagents such as 4-dimethylaminopyridine (DMAP).

EXAMPLE 1

This example illustrates the synthesis of C_2 (see Figure 2).

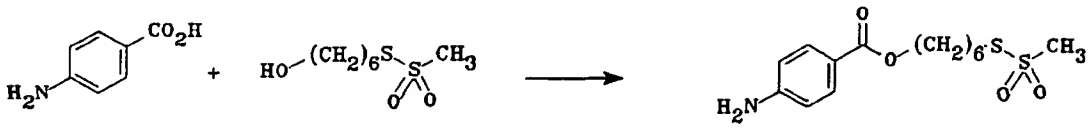


A solution of *p*-aminobenzoic acid (1.32 g; 9.6 mmol), 2-hydroxyethyl methanethiosulfonate (1 g; 6.4 mmol, prepared according to Boldyrev, *et al.*, *Zh. Organic. Khim.* **3**:37 (1967)), DCC (1.98 g; 9.6 mmol) and DMAP (61 mg; 0.5 mmol) in DMF (15 mL) was stirred at room temperature for 5 h. It was then filtered, evaporated, and allowed to stand at room temperature for 2 days. To this was added silica gel (8 g) and the mixture was evaporated to dryness. The residual powder was applied to a column of silica gel, which was eluted with CH_2Cl_2/methanol (97:3). The fractions containing product were combined, and evaporated to dryness. Crystallization from ethyl acetate afforded **C_2** as a white crystalline solid (670 mg; 2.44 mmol; 38% yield) with m.p. of 112-113° C. ^1H NMR. (500 MHz $(CD_3)_2SO$) δ7.63 (d, J=8.7 Hz, 2H), 6.56 (d, J=8.7

Hz, 2H), 6.00 (s, 2H), 4.44 (t, J=6.1 Hz, 2H); 3.56 (t, J=6.1 Hz, 2H), 3.56 (s, 3H). MS (NH_3 DCI): m/z 293 [$(M+NH_4)O^+$, 84%], 276 [$(M\&H)^+$, 10%], 120 [100%].

EXAMPLE 2

This example illustrates the synthesis of C_6 (see Figure 2).



2.1 *Preparation of 6-hydroxyhexyl methanethiosulfonate*

A mixture of 6-bromo-1-hexanol (2.4 g; 0.013 mol) and sodium methane thiosulfonate[2] (1.95 g; 0.0146 mol) in ethanol (30 mL) was heated at reflux for 20 h. It was filtered, and 5 g silica gel was added to the filtrate, which was then evaporated to dryness. The residual powder was applied to a column of silica gel, which was eluted with ethyl acetate/hexane (7.5:2.5). The fractions containing product were combined and evaporated to dryness, to give 6-hydroxyhexyl methanethiosulfonate as a colorless oil (1.64 g; 8.0 mmol; 61% yield).

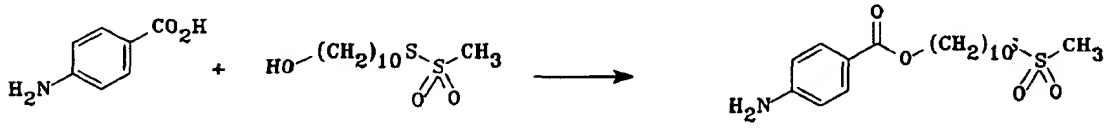
2.2 *Preparation of Targeted Drug C_6*

A mixture of p-aminobenzoic acid (1.11 g; 8.13 mmol), 6-hydroxyhexyl methanethiosulfonate (1.15 mg; 5.42 mmol), DCC (1.68 g; 8.13 mmol) and DMAP (66 mg; 0.50 mmol) in methylene chloride (50 mL) was stirred at room temperature overnight. The mixture was filtered and evaporated. The residue was allowed to sit at room temperature for 1 day, then dissolved in methanol. To this was added 8 g silica gel, and the mixture was evaporated to dryness. The residual powder was applied to a column of silica gel (150 g), which was

eluted with CH_2Cl_2/methanol 9.75:0.25. The fractions containing product were combined, evaporated to dryness, and crystallized from ethyl acetate hexane to afford **3** as a white crystalline solid (280 mg; 0.845 mmol; 10% yield) with m.p. of 85-86°C. ^1H NMR (500 MHz $(CD_3)_2SO$) δ7.62 (d, J=8.7 Hz, 2H), 6.55 (d, J=8.7 Hz, 2H), 5.92 (s, 1H), 4.14 (t, J=6.5 Hz, 2H); 3.49 (s, 3 H), 3.19 (t, J=7.3 Hz, 2H), 1.72-1.64 (m, 4H), 1.41-1.39 (m, 2H). MS (NH_3 DCI): m/z 349 [$(M+NH_4)^+$, 32%], 332 [$(M\&H)^+$, 66%], 120 [100%].

EXAMPLE 3

This example illustrates the synthesis of C_{10} (see Figure 2).



3.1 Preparation of 10-hydroxydecyl methanethiosulfonate

A mixture of 6-bromo-1-decanol (930 mg; 3.42 mmol) and sodium methane thiosulfonate (735 mg; 5.5 mol) in DMF (10 mL) was stirred at room temperature for 3.5 days. The solvent was evaporated, the residue dissolved in methanol, 3 g silica gel was added, and the mixture evaporated to dryness. The residual powder was applied to a column of silica gel, which was eluted with ethyl acetate/hexane (4:6). The fractions containing product were combined, evaporated to dryness, to give 10-hydroxydecyl methanethiosulfonate as a white solid (900 mg; 3.35 mmol; 85% yield).

3.2 Preparation of Targeted Drug C_{10}

A mixture of *p*-aminobenzoic acid (650 mg; 4.75 mmol), 10-hydroxydecyl methanethiosulfonate (850 mg; 3.17 mmol); DCC (980 mg; 4.75 mmol) and DMAP (31 mg; 0.25 mmol) in

methylene chloride (40 mL) was stirred at room temperature overnight. The mixture was filtered and evaporated. To this was added 6 g silica gel and the mixture was evaporated to dryness. The residual powder was applied to a column of silica gel (80 g), which was eluted with CH_2Cl_2/methanol 9.75:0.25. The fractions containing product were combined, evaporated to dryness, and crystallized from ethyl acetate hexane to afford **2** as a white crystalline solid (530 mg; 1.37 mmol; 43% yield) with m.p. of 73-74° C. ^{1}H NMR (500 MHz $(CD_3)_2SO$) δ7.61 (d, J=8.7 Hz, 2H), 6.55 (d, J=8.7 Hz, 2H), 5.91 (s, 1H), 4.13 (t, J=6.6 Hz, 2H); 3.49 (s, 3 H), 3.17 (t, J=7.4 Hz, 2H), 1.68-1.62 (m, 4H), 1.35-1.26 (m, 12H). MS (NH_3 DCI): m/z 405 [$(M+NH_4)^+$, 7%], 388 [$(M\&H)^+$, 66%], 120 [100%].

EXAMPLE 4

This example illustrates the synthesis of T_2 (see Figure 2).

4.1 Preparation of T_2

To a solution of 2,6-dimethylaniline (8.5 g; 70 mmol) and bromoacetic acid (9.75 g; 70 mmol) in dry CH_2Cl_2 (400 mL) cooled in an ice bath was added DCC (14.5 g; 70 mmol) in portions over 30 min. The mixture was stirred and allowed to warm to room temperature overnight.

The product can be isolated and treated with 2-aminoethyl methanethiosulfonate to produce T_2.

EXAMPLE 5

This example illustrates the evaluation of the site-specific therapeutic agents prepared in Examples 1-4.

5.1 Methods

Sit -Dir cted Mutagenesis

Site-directed mutagenesis of the rat skeletal muscle sodium channel (μ1-2) (Trimmer, *et al.*, 1989) was performed to create the Y401C constructs. The mutations were introduced into a 2.5 kb *SphI-KpnI* cassette subcloned into pGEM7 (Promega, Madison, WI) using the oligonucleotide containing the appropriate base substitution.

Expression of Sodium Channels in *Xenopus* Oocytes

Oocytes were removed from adult female *Xenopus laevis* frogs (NASCO, Fort Atkinson, WI; XENOPUS, Ann Arbor, MI) anesthetized by immersion for 10-25 minutes in a 0.25% solution of tricaine (Sigma Chemical Co., St. Louis, MO) in tap water. The 5-fold excess of β subunits compared to a subunits was used to minimize the altered gating properties of Na^+ channels when expressed in oocytes (Isom, *et al.*, 1992; Ji, Sun, George, Horn & Barchi, 1994). Injected oocytes were incubated at 22° C for 24-48 hr prior to recording.

Electrophysiological Recording

For oocyte recordings whole-cell currents were recorded at room temperature (approximately 23° C) using two-electrode voltage-clamp techniques with 3 M Kcl in the pipette and a bath solution containing (mM): 96 NaCl, 4 KCl, 1 MgCl2, 1 $CaCl_2$ and 10 HEPES (pH = 7.6, NaOH). Electrode pipettes were fabricated from 1.2 mm outer diameter thin-walled borosilicate glass (TW120F-6, World Precision Instruments Inc., Sarasota, FL) pulled on a Sutter puller (model P-87). Pipette tips were broken to a diameter of approximately 0.1 mm and plugged with agar made with 3 M KCl for a final resistance of 1-4 MΩ. Electrode series resistance were compensated by the circuitry of the amplifier (Oocyte Clamp OC-725A, Warner Instruments Inc., Hampden, CT, USA). Leak subtraction was

accomplished using a P/8 protocol from a holding potential of -120 mV. Currents were filtered at 2 kHz and digitized at 10 kHz using an IBM-compatible computer. Warner analog-digital interface (model PP-50 Lab1), and custom acquisition software. In order to minimize difficulties associated with adequately voltage-clamping oocytes which expressed large numbers of channels, whole-cell recordings were limited to oocytes expressing less than 5 μAmps of peak current.

Similar recording methods were used in isolated rat ventricular myocytes using the patch-clamp recording technique.

The local anesthetic drugs were introduced at the desired concentration in ND96 to the bath by perfusion with at least 30 ml (bath volume $\simeq$ 0.6 ml). Total bath exchange took less than 3 minutes and the drug was allowed to equilibrate with the oocyte for a minimum of 6 minutes prior to recording.

Voltage Protocols

Current-voltage relationships were produced by stepping the membrane potential from -60 to +50 mV by increments of 5 mV from a holding potential of -80 mV. A repetition frequency of 01.2 Hz was used for such voltage families. Steady-state activation curves of whole-cell currents were calculated from the current-voltage relationships by scaling the peak currents by the net driving force (i.e., $V-E_{rev}$) using the equation $g = I/(V-E_{rev})$ where E_{rev} = +45 to +60 mV. Steady-state fast-inactivation curves were constructed by normalizing the current recorded in test pulses to -10 mV following 50 msec prepulses to voltages ranging from -100 to -10mV. A repetition frequency of 0.2 Hz was used for the steady-state inactivation protocol. Recovery from inactivation was assessed using a two-pulse protocol, in which identical depolarizing voltage pulses to -20 mV for 50 msec were applied to oocytes before (conditioning) and after (test) a variable duration repolarization to -80 mV (recovery potential). The peak current evoked by the second (test) depolarization was normalized to amplitude of the first

(conditioning) depolarization and represents the fraction of channels that had recovered from inactivation during the interpulse recovery period.

5.2 *Evaluation of Site-Specific Agents*

Preliminary studies have been performed on four prototypes of the drug illustrated in Figure 1B. The chemical structures of these agents are shown in Figure 2. Studies using these drugs were performed on three distinct types of Na^+ channels: skeletal muscle Na^+ channels expressed in *Xenopus sp.* oocytes, native heart Na^+ channels in isolated rat ventricular myocytes and mutated skeletal muscle Na^+ channels (i.e., Y401C) in which the naturally occurring tyrosine at position 401 was replaced with cysteine (i.e., the residue naturally found in heart). Below, we will refer to native heart Na^+ channels and Y401C channels as "heart-like" Na^+ channels.

Results with C_2

Application of C_2, at concentrations between 100 μM and 1 mM, cause decreases in whole-cell Na^+ current, enhanced rate of whole-cell Na^+ current decay following channel activation, leftward shifts in the steady-state inactivation curve and slowing of recovery from inactivation. These effects of C_2 on Na^+ channels are the hallmarks of nonpolar group 1b agents represented by benzocaine. However, the effects of C_2 on heart and Y401C Na^+ channels (i.e., heart-like Na^+ channels) were distinct from their effects on skeletal muscle Na^+ channels. Specifically, when the drug is present in the solution bathing the Na^+ channels, Na^+ channel properties are modified as expected in the presence of class 1b antiarrhythmic. However, when the drug is applied to native Na^+ channels the functional changes are <u>not reversible</u> following washout of the drug. In other words, the drug's effects on Na^+ channel function remained after the C_2 was removed from the bathing solution. The inability to wash out

the effects of C_2 was also observed in Y401C channels. This establishes three facts. First, on the time scale of the experiments (i.e., 20-30 minutes), C_2 has been permanently attached to the channel. Second, the effect depends on the presence of the cardiac-specific cysteine within the pore. Third, the attachment site of the drug in the pore (via the sulfhydryl reactive anchor) is sufficiently close to the local anesthetic binding site to allow us to observe the expected modification of the Na^+ channels.

Further proof that the mode of action of these compounds involves the unique cysteine in the pore of heart-like Na^+ channels was established by examining the effects of Cd^{2+} and dithiothreitol (DTT). Specifically, the application of DTT totally reversed the effects of C_2 observed following washout of drug from the bath. This is expected if the drug is linked via a disulfide bond. Second, following C_2 application (after washout), the Na^+ current is no longer blocked by μM amounts of Cd^{2+}; Cd^{2+} binds with very high affinity to free sulfhydryls, but not disulfide linked sulfhydryls. As expected, if the cysteine in the pore has reacted with the drug to form a disulfide link, the channel will no longer bind Cd^{2+} thereby becoming resistant to block. Following DTT application, C_2 modified channels restore their sensitivity to Cd^{2+} and this restoration occurs simultaneous with the reversal of the local anesthetic effects.

Results using C_6, C_{10} and T_2

Similar results have been obtained with C_6, C_{10} and T_2. First, C_6 is far more potent than C_2 which we believe is related to a more favorable length of the linker thereby allowing a more optimal interaction of the active agent with the local anesthetic binding site. These results suggest that manipulation of the linker length can be used to control the potency of the drug. Second, T_2 shows one additional (expected) property not observed for the C_X compounds. Specifically, we observe that the amount of Na^+ channel block by this agent depends on the frequency of stimulation of Na^+

channels (i.e., the block is use-dependent). Since T_2 is a polar local anesthetic, this property was fully expected and predicted. This further confirms that these agents are working in the designed manner: the drug is anchored in the channel pore and delivers that active agent to the local anesthetic binding site.

All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A compound represented by the formula:

A—L—D

wherein

A is an anchoring moiety;

L is a linking group; and

D is a drug.

2. A compound in accordance with claim 1, wherein said anchoring moiety is a functional group capable of covalent attachment to a target site.

3. A compound in accordance with claim 1, wherein said anchoring moiety is a non-peptide affinity ligand for a target site.

4. A compound in accordance with claim 1, wherein said anchoring moiety is a sulfhydryl-reactive group.

5. A compound in accordance with claim 4, wherein said sulfhydryl-reactive group is a member selected from the group consisting of methanethiosulfonate esters, dithiopyridyl groups, cystine and maleimide.

6. A compound in accordance with claim 3, wherein said non-peptide affinity ligand has a reactive functional moiety selected from the group consisting of α-diazo ketones, α-halo ketones, pentafluorophenyl esters, and 2,4-dinitrophenyl esters.

7. A method for the localization of a drug at a preselected target site, comprising administering to a host, a compound represented by the formula:

A—L—D

wherein

A is an anchoring moiety;

L is a linking group; and

D is a drug,
wherein either A or D or both are specific for said preselected target site.

8. A method in accordance with claim 7, wherein said anchoring moiety is a functional group capable of covalent attachment to a target site.

9. A method in accordance with claim 7, wherein said anchoring moiety is a non-peptide affinity ligand for a target site.

10. A method in accordance with claim 7, wherein said anchoring moiety is a sulfhydryl-reactive group.

11. A method in accordance with claim 10, wherein said sulfhydryl-reactive group is a member selected from the group consisting of methanethiosulfonate esters, dithiopyridyl groups, cystine and maleimide.

12. A method in accordance with claim 9, wherein said non-peptide affinity ligand has a reactive functional moiety selected from the group consisting of α-diazo ketones, α-halo ketones, pentafluorophenyl esters, maleimide and 2,4-dinitrophenylesters.

ORALLY ACTIVE SITE-SPECIFIC DRUG DELIVERY

ABSTRACT OF THE DISCLOSURE

Compounds and methods which are useful for the site-specific delivery and localization of drugs are provided. The compounds can be represented by the formula:

A–L–D

wherein A is an anchoring moiety; L is a linking group; and D is a drug.

Figure 1

a)

"Drug" = A + L + D Target = Receptor Drug + Target

A = Anchor ≡ [A]
L = Linker ≡ ~~~
D = Active Agent = [D]

b)

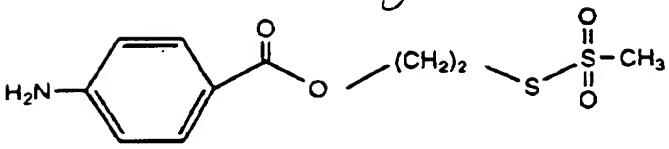
Drug = Sulfhydryl reactive + Linker + Local Anesthetic

Na^+ Channel

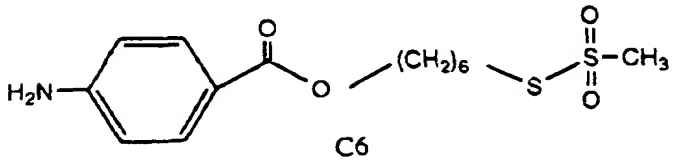
Drug + Na^+ Channel.

Sulfhydryl reactive ≡ [S⁻]
Linker ≡ ~~~
Local Anesthetic ≡ [~~~]

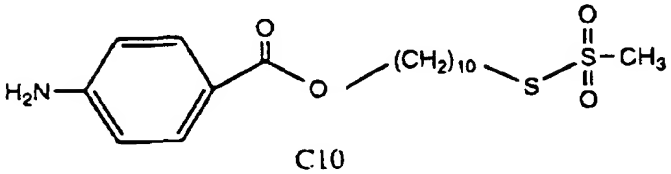
Figure 2



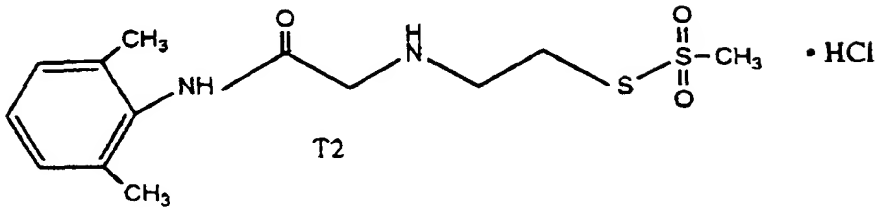
C2



C6



C10



T2